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Constituents of the Leaves of *Odontioda* Marie Noel 'Velano' with Inhibitory Activity on RANKL-induced Osteoclast Differentiation

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Four compounds (**1-4**) were isolated from the leaves of *Odontioda* Marie Noel 'Velano'. **2-4** were identified as phenanthrene derivatives with spectroscopic analysis. **2** reported here was isolated for the first time from this plant. Among them, **2** and **3** potently inhibited the receptor activator of nuclear factor-κB ligand (RANKL) induced osteoclast differentiation.

Keywords: *Odontioda* Marie Noel 'Velano'; phenanthrene; osteoclast differentiation

Bone disease such as osteoporosis caused by aging is a serious problem in our country, faced with an aging society, and development of therapeutic drugs preventing and/or treating osteoporosis should be urgent. Therefore, we performed the search for natural materials having inhibitory activity on osteoclast differentiation to develop new drugs. Intervention in osteoclast differentiation is considered an effective therapeutic approach for the treatment of bone disease. A screening test conducted by us led us to find the methanolic extract of *Odontioda* Marie Noel 'Velano' as an active material reducing RANKL-induced osteoclast differentiation of RAW264.7 cells. Thus, we have attempted to isolate active components from the extract. *O.* Marie Noel 'Velano' (Orchidaceae) is an artificial insemination of *Odontoglossum* and *Cochilida. O.* Marie Noel 'Velano' is mainly cultivated as an ornamental plant.

We have recently reported that the flavone glycosides and flavanone glycosides isolated from leaves of this plant have scavenging activity against the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical.[1)]

As a result of preparative fractionation of the methanol extract of leaves of *O.* Marie Noel 'Velano', four known compounds were isolated, some of which showed inhibitory effect on osteoclast differentiation induced by RANKL.

Leaves of *O.* Marie Noel 'Velano' were purchased from the Orchid Garden Co., Ltd., Nagano Prefecture, Japan, in April 2009. This specimen was verified and identified by Mr. H. Sumiyoshi (Orchid Garden Co., Ltd.) and a voucher specimen (#20090617) was deposited in the Medicinal Plant Garden of Josai University.

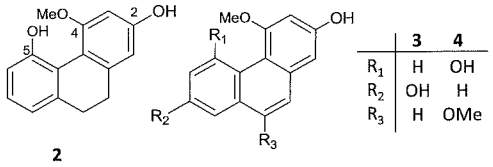
The leaves of *O.* Marie Noel 'Velano' (2.3 kg) were extracted with MeOH two times under reflux for 2 hrs. The MeOH extract was concentrated under reduced pressure, and the concentrate portion (26 g) was passed through a Diaion HP-20 column (H_2O→ 50%MeOH→ MeOH). The MeOH elute fraction (7.8 g) was chromatographed on a silica gel column to give nineteen fractions. β-Sitosterol (**1**, 269 mg)[2)] was deposited from fr. 8. Fr. 4 was chromatographed on a Sephadex LH-20 column and purified by

Table 1. ^{1}H-NMR (400MHz) spectral data for **2** in CD_3OD, **3** in acetone $-d_6$, **4** in $CDCl_3$.

	2	3	4
H-1	6.50 (1H, d, 2.4)	6.70 (1H, d, 2.4)	6.92 (1H, s-like)
H-3	6.54 (1H, d, 2.4)	7.08 (1H, d, 2.4)	6.72 (1H, s-like)
H-5		7.42 (1H, d, 6.7)	
H-6	6.81 (overlapped with signal at H-8)	7.10 (1H, dd, 2.6, 6.7)	7.24 (1H, dd, 1.1, 7.6)
H-7	7.06 (1H, dd, 7.6, 7.8)		7.49 (1H, dd, 7.6, 7.6)
H-8	6.81 (overlapped with signal at H-6)	7.42 (overlapped with signal at H-5)	7.94 (1H, d-like, 7.6)
H-9	2.61 (2H, m)	7.64 (1H, d, 8.8)	
H-10	2.61 (2H, m)	7.51 (1H, d, 8.8)	6.74 (1H, s)
5-OH			9.56 (1H, br s)
7-OH		9.51 (1H, br s)	
4-OMe	3.93 (3H, s)	4.16 (1H, 3H, s)	4.04* (3H, s)
9-OMe			4.05* (3H, s)

Coupling constants (J in Hz) were given in parentheses. Chemical shifts were expressed in δ (ppm) refering to solvent signal.
* : maybe interchanged.

reversed phase HPLC to obtain hircinol (**2**, 1.1 mg),[3] flavanthrinin (**3**, 3.5 mg)[4] and 2,5-dihydroxy-4,9-dimethoxyphenanthrene (**4**, 1.5 mg).[5] These isolated compounds were identified by comparison of NMR spectral data including MS and other spectroscopic data to literature values.[3-5] The ^{1}H- and ^{13}C-NMR spectral data of compounds **2** - **4** are listed in Table 1 and 2, respectively. **2** reported here was isolated for the first time from *O.* Marie Noel 'Velano'. Inhibitory activity of these isolates (**1** - **4**) and linarin, which was isolated from this plant previously[1], on RANKL-induced osteoclast differentiation was examined according to published methods.[6] Tartrate-resistant acid phosphatase (TRAP) activities of medium treated with isolated compounds were evaluated relatively as compared to TRAP activity of medium treated with RANKL only as a control. The ratio of osteoclast differentiation of treatment with isolated components at 5 and 50μM were expressed versus control, the value of which was taken as 100. As the results, even though none of the test compounds showed inhibitory activity on 5μM, **2** and **3** potently inhibited the RANKL-induced osteoclast differentiation on 50μM. Osteoclast differentiation ratios of **2** and **3** were <0.01 and 0.08 respectively. Furthermore, the cytotoxicity of each compound at 50μM was not shown on microscopic observation of TRAP staining.



	3	4
R_1	H	OH
R_2	OH	H
R_3	H	OMe

2

Fig. 1. Structures of isolated compounds **2**-**4**.

Table 2. ^{13}C-NMR (100MHz) spectral data for **2** in CD_3OD, **3** in acetone $-d_6$, **4** in $CDCl_3$.

	2	3	4
C-1	110.2	108.2	106.6
C-2	159.1	157.8	153.9
C-3	100.3	103.0	99.9
C-4	157.0	156.8	155.6
C-4a	115.2	114.4	110.1
C-4b	122.2	120.3	120.1
C-5	154.6	127.9	152.1
C-6	118.4	117.5	117.3
C-7	128.5	155.7	126.9
C-8	120.8	121.5	114.1
C-8a	142.0	135.5	132.1
C-9	32.5	130.2	154.5
C-10	32.2	127.5	101.9
C-10a	144.8	137.6	136.9
4-OMe	57.5	59.0	58.5
9-OMe			55.7

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